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FINAL REPORT

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**INVESTIGATION OF TIP GRAFTING FOR EARLY EVALUATION OF
FRUIT QUALITY AND COLD HARDINESS IN BREEDING TREE FRUITS
FOR THE PRAIRIE**

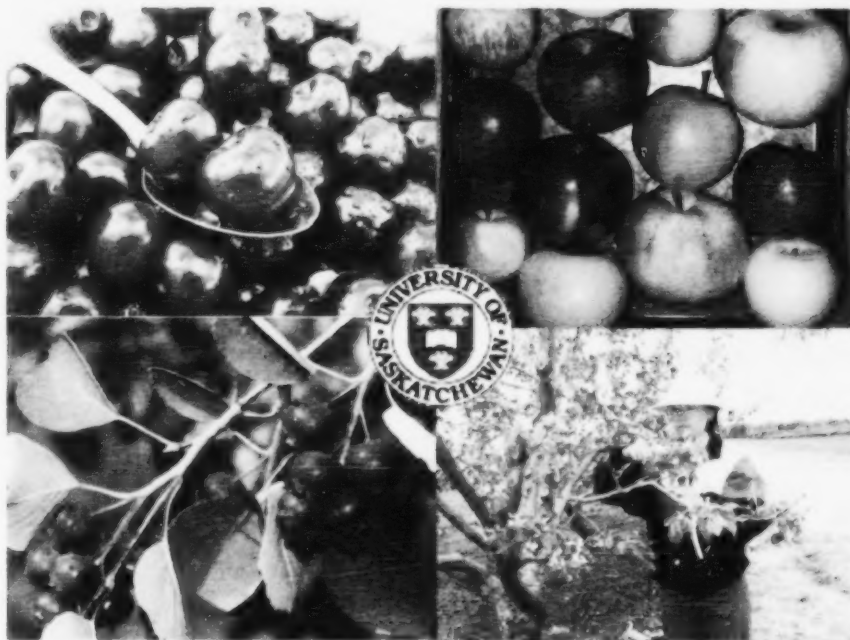
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**Final Report
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**Investigation of tip grafting for evaluation of cold
hardiness and juvenility in tree fruit breeding**



**Funded by:
Agriculture Development Fund (ADF)**

**Report Prepared by:
Qiuju Lu and Bob Bors**

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INTRODUCTION

Project summary

The goal of this project was to investigate the influence of tip grafting on the juvenility and cold hardiness of scions. It was hoped that tip grafting could greatly reduce costs associated with fruit breeding by hasten fruiting of seedlings and reducing space required for the breeding program. Tip grafting had never been tried in such a cold region as the Canadian prairies, so it was unknown if this technique would be a viable alternative to traditional methods. The tested species included apples, sour cherries, and saskatoons.

The results in this study showed tip grafting increased the cold hardiness of scions to a much greater extent than anticipated. This would indicate that selection for cold hardiness should not be done on tip grafted trees and thus addition screening of a modification of the system developed would need to occur. Tip grafting may be used maintain non-hardy germplasm or hybrids that are the results of hardy parents crossed to non-hardy parents.

Juvenile seedlings of Saskatoon berry (*Amelanchier alnifolia* Nutt) and sour cherry hybrids (*Prunus cerasus* x *P. fruticosa*) were chip-budded onto the tips of branches of mature, bearing trees at the University of Saskatchewan field plot. After two growing seasons, tip grafting on sour cherry seedlings had decreased flowering percent by 69.7%, leaf number by 64%, shoot length by 84%, and shoot diameter by 76% compared with scion controls. By August 12, terminal growth of tip grafted branches had ceased but seedlings were still actively growing. By the end of October, leaf retention was higher in seedlings. Only 0.3% of the tip grafted branches showed winter damage, versus 69.2% of the scion controls had winter dieback. This study showed tip grafting promoted cold acclimatization and hardiness of cherries. Tip grafting on Saskatoon berry seedlings had increased flowering compared with scion controls. Tip grafting increased leaf number by 93%, shoot length by 257% and shoot diameter by 42%. Due to the cold hardy character of saskatoons, tip grafting in this species had little effect on terminal bud formation, leaf retention and cold hardiness.

In order to determine the physiological indicators for phase change in tree fruit breeding, scions from different developmental zones of apple seedlings were chip-budded onto the tip of the branches of mature, bearing 'Selin' crabapple trees. The results showed that leaf number, shoot length, thorn number and total lateral shoot number were negatively, and leaf number to shoot length ratio and flower number were positively related to maturity levels. The thorn number, lateral shoot number, shoot length, leaf number to shoot length ratio related to juvenility could be used as physiological or morphological markers for phase change. Terminal bud cession occurred earlier in adult tip grafts. Leaf retention was only significantly different among genotypes, and the difference among genotypes on leaf retention was consistent in different years. Flowering only happened in tip grafts derived from the scions above

genotypes, and the difference among genotypes on leaf retention was consistent in different years. Flowering only happened in tip grafts derived from the scions above 1.0m on apple seedlings, and the transitional zone in apple tip grafts derived from seedlings was between 1.0m and 2.0m, which was lower than that on self-rooted apple seedlings. These results suggested that tip grafting for apple seedlings could enhance maturation, thereby shortening the juvenile period, only if the scions were in transitional phase.

Project Personnel

Dr. Bob Bors is head of the project with Rick Sawatzky assisting in maintaining the plants.

Qiuju Lu is a graduate student working on her MS degree. This report is abridged from her thesis.

Rationale

In order to develop tree fruit crops for these areas, breeding and selection for improved winter survival has received a great deal of attention. With several breeding generations, winter survival has been gradually improved and development of advanced varieties with good fruit quality has occurred (Bors, 2003). The cost for new variety development, however, is very high in most tree fruit crops especially those belonging to the Rosaceae family due to long juvenile periods and large tree size (Hansche, 1983).

Shortening the juvenile period can reduce the selection cycle and thereby decrease the associated breeding costs. Breeders have tried many methods to accelerate flowering, in order to obtain an earlier assessment of new seedlings. These methods include: bark girdling of the tree trunk, pruning of lateral branches, pruning of the roots, reorienting branches horizontally, knotting of the stems, and grafting of inverted bark rings on the trunk or branches. None of these methods are capable of reducing the juvenile period if used on very young seedlings (Brown, 1975; Sherman and Lyrene, 1983).

Shortening of the juvenile period by grafting seedling scions onto the tip of the branches of mature cherry trees (tip grafting) was reported by Burbank (1921). The tip grafted cherry scions bore fruit in the second season after grafting (Burbank 1921). It is possible that this grafting method allowed the scions to transfer from a juvenile phase to an adult phase. Unfortunately, the tip grafted shoots were not compared to the control seedlings, nor was the age of the scions reported, so this claim cannot be substantiated.

A reduction in tree size is another way the cost to both breeders and producers could be improved. A reduction in mature tree size would directly correlate to land requirements and yield per acre. Grafting of seedling scions onto dwarf rootstocks has been used successfully in apples to reduce the size of the trees (Zimmerman, 1972). However, for handling seedling population, tip grafting is more efficient and superior at reducing the land required as reported by Sherman and Lyrene (1983) in their pecan

breeding programs. Burbank (1921) grafted more than 500 cherry scions onto one single mature tree to simultaneously test all of the seedlings. The technique of tip grafting was developed for fruit trees in substantially warmer climates than the Canadian prairies, and the impact of extreme cold on the tip-grafts is unknown. If tip grafting results in the improved cold hardiness of scions, then the tip grafting system would be useful in the conservation of breeding materials. If tip grafting reduces cold hardiness, the tip grafting technique could allow early evaluation of cold hardiness.

Graft transmission from rootstock to scion has been reported in many plants for a variety of traits including: branching in poinsettia (Dole and Wilkins, 1992); virus resistance in tobacco (Smirnov et al., 1997) and in sweet potato (Okada et al., 2001); phytochrome-sensitive flowering in peas (Weller et al., 1997); and phloem protein transportation in heterografts of Cucurbitaceae (Golecki et al., 1998). A few reports have shown that top working enhanced the cold hardiness of apple scions (Patterson, 1936; Blair, 1952). Top working is grafting scions onto the limb of established trees; versus tip grafting is grafting scions onto the branches of established trees. Therefore, tip grafting can also result in the improvement of cold hardiness of scions. The mechanism of tip grafting on this improvement is probably through cold acclimation.

Cold acclimation of woody perennials is a very important first step for winter survival. It begins soon after both the cessation of vegetative growth and the beginning of terminal bud formation (Hurme et al., 1997). In most woody perennials, the first stage of cold acclimation is induced by the shortening of the photoperiod, which leads to growth cessation. Low night temperatures then trigger the second stage of cold acclimation and this transition from the first to the second stage of acclimation generally happens during leaf fall (Weiser, 1970; Westwood, 1993). The ability of the rootstocks to cease the vegetative growth of scions before early frost and induce a timely leaf drop in early winter is a desirable index of cold hardiness. If there is a correlation among the timing of terminal bud formation, leaf drop and cold hardiness, bud phenology can then be used as a physiological marker for the early evaluation of cold hardiness.

Goals

- 1) To investigate the influence of tip grafting on vegetative growth and maturity and cold hardiness in apple trees.
- 2) To investigate the influence of tip grafting on vegetative growth and maturity and juvenility on sour cherries and saskatoons.
- 3) To compare the vegetative growth, juvenility and cold hardiness between juvenile and adult tip grafts.

RESEARCH SECTION

1.0 Growing conditions

This project was conducted at the Horticulture Field Lab of the University of Saskatchewan in Saskatoon (52°10' North, 106°41' West), Saskatchewan, Canada. Soil type at the field site was a Dark Brown Chernozem clay loam. Daily maximum and minimum air temperatures and precipitation during the study period from May 2000 to May 2003 were obtained from University of Saskatchewan, Kernen Research Weather Station located approximately 3km from the experimental site (Figure 1.1).

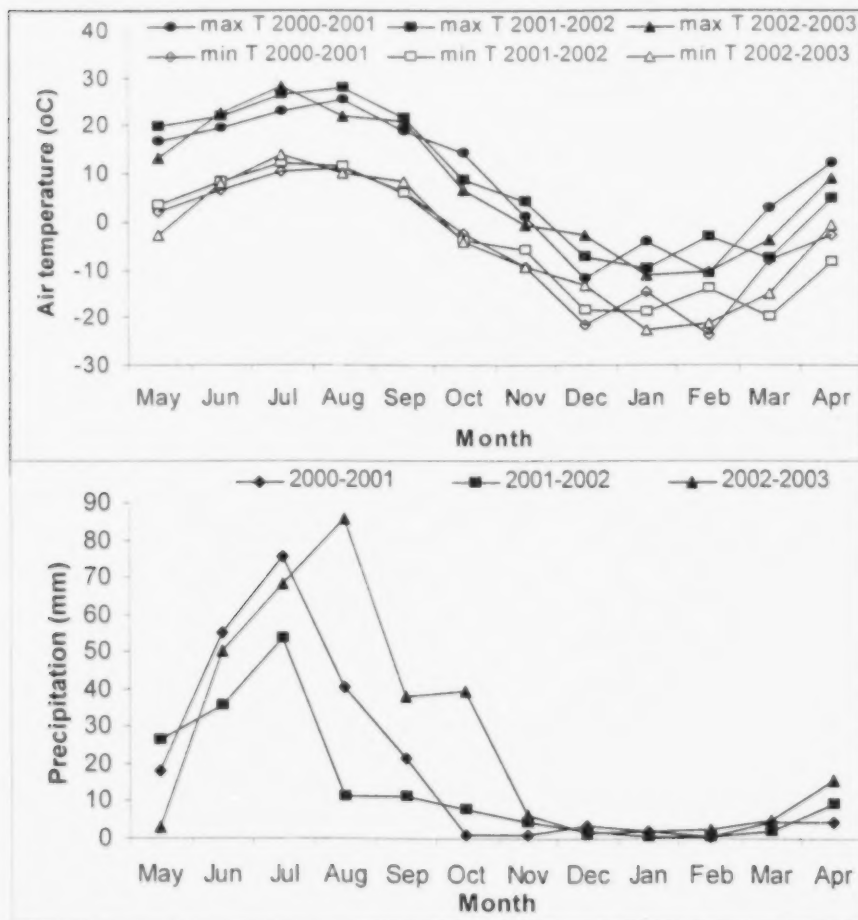


Figure 1.1 Average maximum and minimum air temperatures and precipitation from May 1st to April 30th during the study period (2002-2003) in Saskatoon, SK, Canada.

The first frost occurred on September 23, September 12, and September 24 in 2000, 2001, and 2002, respectively. The minimum temperatures were -34.77°C in February, -35.12°C in January, and -38.41°C in March for the year 2001, 2002, and 2003,

respectively. The long-term average precipitation is approximate 303 mm. The year 2000-2001 had total 226 mm precipitation, while the year 2001-2002 was pretty dry with only 164.4 mm precipitation.

2.0 Choosing the tip grafting method

Four techniques were compared for tip grafting purposes (Table 2.1). While each method has traditionally been used for tree fruits, usually large branches are involved; tip grafting uses the ends of the youngest branches. Saskatoon and sour cherry branches are very thin at the ends and the wood is rather hard and brittle which made chip budding the only viable option. Apples, which have thicker and more succulent branches, were best done with the cleft grafting or chip budding techniques. As chip budding appeared to be equally good in either May or August it was chosen over cleft grafting for this study.

While apples and saskatoons had greater than 95% bud take, cherries were the worst with 70% bud take. At a recent cherry symposium it was noted that cherries often have graft incompatibility problems so it may be a characteristic of this species.

Qiuju used parafilm instead of traditional method using rubber bands and grafting compound. Parafilm is much easier to use, does not create a mess, and does not have to be removed because buds can grow through it (Figure 2.1). Consequently, we have switched to parafilm for most of our grafting in our breeding program and include teaching this method in our grafting seminars and classes.

Table 2.1. Comparison of four grafting techniques for tip grafting.

Grafting technique	Season	Apples	Cherries	Saskatoons
Cleft grafting	May	good	tip branches too small	tip branches too small
Whip-and-tongue	May	hard to do, low survival	tip branches too small	tip branches too small
Chip budding	May or August	good	ok	good
T-budding	August	hard to do	tip branches too small	tip branches too small

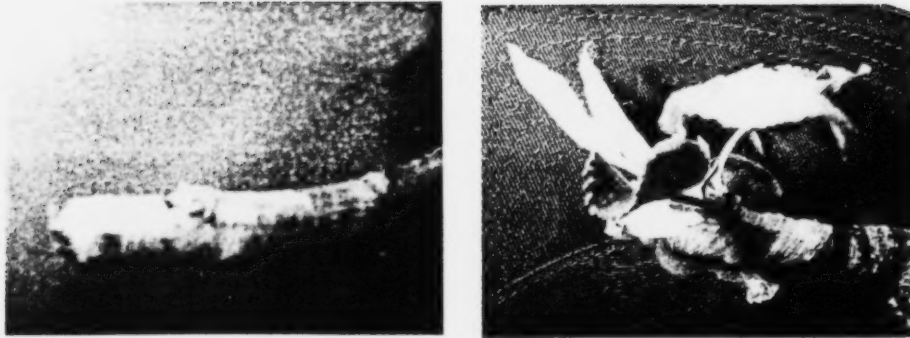


Figure 2.1. A newly grafted chip bud and one with a shoot that has grown through the parafilm.

3.0 Investigation of tip grafting on cold hardiness in apple trees

Introduction

Tip grafting is grafting buds onto the tips of branches of mature trees; versus basal grafting is the traditional method of grafting buds onto the base of young rootstocks. Tip grafting onto mature trees (Sherman and Lyrene, 1983) and basal grafting onto dwarf rootstocks (Zimmerman, 1972) have been used to save space in handling seedling populations. Burbank (1921) tip grafted more than 500 cherry seedlings onto one single mature tree to simultaneously test all the seedlings. This method has been used in pecan breeding programs (Sherman and Lyrene, 1983). Tip grafting could be particularly valuable for the apple breeding program in Saskatchewan, Canada since only 'Ottawa 3' is the only dwarfing rootstocks recommended for trial in our province. Since development and use of tip grafting occurred in climates much milder than the Canadian prairies, this study was initiated to determine the feasibility of using tip grafting in our region by comparing it to the next best alternative of grafting onto 'Ottawa 3'.

An ideal system for managing seedling populations would provide natural or artificial screening for cold hardiness (Sherman and Lyrene, 1983), especially in breeding programs north or in higher altitudes of normal production areas. Rootstocks influenced the cold hardiness of cherry (Palonen and Buszard, 1997) and peach scions (Layne, 1994; Layne and Jui, 1994). A few reports have shown that top working enhanced the cold hardiness of apple scions (Patterson, 1936; Blair, 1952). It is

hypothesized that tip grafting could result in the improvement of cold hardiness of apple, compared to basal grafting onto dwarf rootstocks. However, it is not clear to what degree the scions would be influenced physiologically by the tree stocks, and which factors related to winter survival will be influenced by the technique.

Terminal growth cessation (Proebsting, 1978; Guak and Fuchigami, 2001; O'Neill et al., 2001) has been used as indicators of cold acclimation and cold hardiness. The first stage of cold acclimation in most woody perennials is induced by shortening photoperiod causing growth cessation and the second stage of cold acclimation is triggered by low night temperature (Weiser, 1970). In the annual growth cycle of woody perennials, cessation of shoot growth is generally concomitant with terminal bud formation (Rinne et al., 1999). Cold acclimation starts after terminal bud formation and cessation of vegetative growth, which is an important first step for winter survival (Hurme et al., 1997). The transition from the first to the second stage of acclimation happens during leaf fall (Weiser, 1970; Westwood, 1993). Maturity judged by the proportion of leaves retained during early winter was closely related to winter injury (Chanasyk, 1953). Leaf retention (Adrichem, 1970; Wood and Reilly, 2001) has also been used as an atypical symptom for cold hardiness. However, other authors indicated that leaf retention was not closely related to cold hardiness (Zatylny et al., 1996). The ability of tip grafting to cease vegetative growth of scions before early frost and induce timely leaf drop in early winter maybe an important index of cold hardiness. If there is close correlation between cold hardiness and timing of terminal bud cessation or leaf drop, terminal bud cessation or leaf drop can be used as physiological markers for early evaluation of cold hardiness.

The objectives of this study were to compare winter dieback of apple scions tip grafted on crabapple tree stocks and basal grafted onto 'Ottawa 3' rootstocks and to investigate the relationship between winter dieback and vegetative growth, growth cessation and leaf retention for these two alternative ways of handling seedling populations.

Materials and methods

Two trees of each four crabapple cultivars, 'Dauphin', 'Garnet', 'Trailman', and 'Fuchsia Girl', were used to receive tip grafting. These eight trees were ten years old, grafted onto Siberian crab seedling rootstocks, showed no signs of winter damage, were of similar size, and were located in the same row. One-year-old 'Ottawa3' was used as a rootstock for basal grafting. 'Golden Delicious', 'Macintosh', and 'SK Prairie Sun' were chosen to represent a range of cold hardiness with 'SK Prairie Sun' cold hardy, 'Macintosh' intermediate and 'Golden Delicious' not cold hardy. 'Ottawa3' and crabapples were self and reciprocally grafted.

In February 2001, budwood of 'Golden Delicious' and 'Macintosh' was obtained from Canadian Clonal Genebank, Harrow, Ontario. Budwood of 'SK Prairie Sun', 'Ottawa3' and crabapple was collected from University of Saskatchewan Orchard. The stems were wrapped with moist paper towel in polyplastic film and stored at 5°C. 'Ottawa3' rootstocks were obtained from Treeco Nurseries (Oregon, USA) in February 2001. The rootstock bundles were mixed with moist sawdust and stored at 5°C until grafting. At the end of April, buds were chip-budded onto 'Ottawa3' rootstocks and kept

in the cooler at 5°C covered with moist sawdust and planted in mid-May, with the basal grafted buds facing the North. In early May, buds were chip budded onto the tip of the branches of crabapple trees, with the tip grafted buds facing up. Budding was done by hand using parafilm to hold buds in place and maintain humidity. The stems above the graft unions were cut for both basal and tip grafting one week after planting.

The experimental design was a split-plot design with four replications, two grafting systems as main plots, five scions as sub-plots. Crabapple cultivars were nested in different reps, so differences between crabapples were not analyzed. For each treatment within a replication, twenty buds were taken alternately from budwood, and chip budded onto crabapple tree stocks or 'Ottawa3' rootstocks.

Data was collected in each year on apical shoots over a two-year period after grafting. Winter survival was calculated as a percent of the previous season's growth that had bud break in late-May. Shoot length and leaf number were measured in mid-September and November, respectively. In the first year, shoot length was measured from the grafted bud scale scar to shoot tip. In the second year, shoot length was measured from previous year's terminal bud scale scar to shoot tip. Cold acclimation factors including terminal bud cessation and leaf drop were assessed. Terminal bud cessation was assessed twice a year, in mid-August and mid-September, around the first frost. Terminal bud cessation was defined as the point where apical meristematic tissues were free of visually recognized leaves, whether bud scales formed or not (Guak and Fuchigami, 2001). Cumulative percentage of terminal bud cessation in each experimental unit was recorded. Leaf number retained on the tree was counted in early-, and late-November, after which most of the leaves had dropped off. Leaf drop is calculated by total leaf number minus leaf retained on the tree and leaf drop percent was calculated.

The SAS program (SAS institute, 1999) was used for statistical analysis. Analysis of variance (ANOVA) was conducted on winter survival and vegetative growth using a split-split-plot model, adding year as a source of variation, in the program of General Linear Model (GLM). When year was significant, data were analyzed separately for each year, using a split-plot model. Percent of terminal bud cessation and leaf drop was subjected to square root arc sine transformation before data analysis. ANOVA on terminal bud cessation and leaf drop was conducted as a split-split-plot model, adding observation time as a source of variation, using the data from each year as a separate variable. Means of treatments were separated using a least square means (lsmeans) multiple comparison procedure.

Linear regression analysis was conducted using winter survival as the dependent variable while vegetative growth, terminal bud cessation, and leaf drop were independent variables. Vegetative growth was based on leaf number, shoot length and leaf number to shoot length ratio. Regression analysis was done separately for each year using the means of each experimental unit.

Results

a. Winter survival

Tip grafting increased winter survival of apple scions dramatically, especially for the cold susceptible cultivars. In general, tip grafts had 37.1% more winter survival than basal grafts (Table 3.1). In basal grafts, winter survival was significantly different among cold hardy and cold sensitive scions. The rank of winter survival of basal grafts was: crabapple > 'SK Prairie Sun' > 'Ottawa3' > 'Macintosh' > 'Golden Delicious' (Table 3.1). Winter survival of tip grafts of 'Golden Delicious' was significantly lower than other scions (Table 3.1). The winter survival of tip grafts was moderately correlated with that of basal grafts ($r=0.5260$, $n=40$, $P=0.0005$).

Table 3.1 Winter survival (%) of apple grafts as affected by grafting system and scions in 2001 and 2002.

Grafting system	Scion					Mean
	crabapple	Ottawa3	Prairie Sun	Macintosh	Golden Delicious	
Basal grafting	84.8 b ^z	67.9 c	76.2 bc	34.6 d	10.5 d	54.8 B ^y
Tip grafting	95.5 a	94.9 a	97.5 a	95.0 a	76.4 bc	91.9 A

^z Mean separation by least square means multiple comparison procedure at $P = 0.05$.

Numbers followed by different letters in lower case were significantly different.

^y Mean separation by least square means multiple comparison procedure at $P = 0.05$.

Numbers followed by different letters in upper case were significantly different.

Autografts of crabapple had more winter survival than autografts of 'Ottawa3' by 27.6% (Table 3.1). When crabapple was grafted onto 'Ottawa3' rootstock, the cold hardiness of crabapple was decreased significantly, versus the cold hardiness of 'Ottawa3' was improved dramatically when 'Ottawa3' was grafted onto crabapple.

b. Terminal bud development and leaf drop

By mid-August of both years, most terminal bud cessation had occurred on tip grafts, versus only 25.9% and 53.3% on basal grafts in 2001 and 2002, respectively (Figure 3.1). By mid-September, almost 100% of terminal bud cessation was achieved on tip grafts, versus 59.6% and 90.3% on basal grafts in 2001 and 2002, respectively. Basal grafts of 'Golden Delicious', the most cold sensitive cultivar, consistently had the lowest percent of terminal bud cessation relative to other cultivars, and 'Macintosh', the second most cold sensitive cultivar, consistently had the second lowest terminal bud cessation in both years (Figure 3.1).

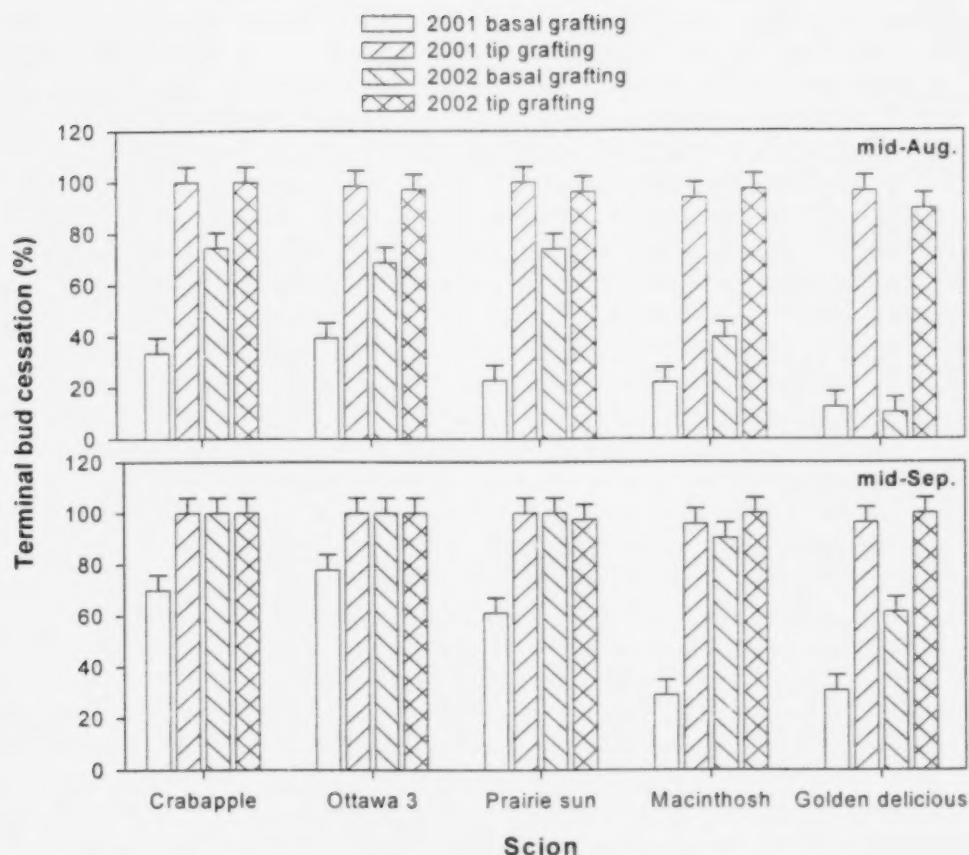


Figure 3.1 Terminal bud cessation (%) of apple grafts as affected by grafting system and scions in mid-August and mid-September in 2001 and 2002. Vertical bars indicate SE.

In early July 2002, most terminal buds of grafts stopped growing on both rootstocks. In early August, terminal buds of basal grafts broke and started to grow again in the current season, while the terminal buds of tip grafts did not. At the end of evaluation period, tip grafts on mature crabapple rootstocks had further terminal bud development compared with the grafts on juvenile Ottawa3 rootstocks for all the five cultivars (Figure 3.2). On both mature crabapple and juvenile Ottawa3 rootstocks, in agreement with the cold hardiness levels, 'Golden delicious' had the lowest and 'Macintosh' had the second lowest terminal bud formation rating.

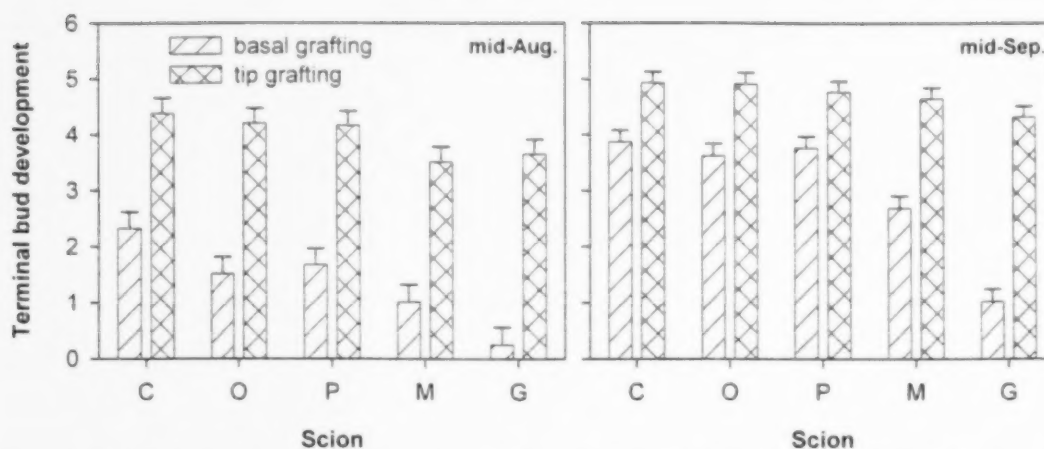


Figure 3.2 Terminal bud development of apple grafts as affected by grafting system and scions in mid-August and mid-September in 2002. Vertical bars indicate SE. C = crabapple, O = 'Ottawa 3', P = 'Prairie sun', M = 'Macintosh', and G = 'Golden delicious'.

Terminal bud cessation and terminal bud development stage was closely related to winter survival in both observation times in 2002 (Table 3.2).

Table 3.2 Estimated correlations (r) between winter survival (%) and various independent variables measured on apical shoots in 2001 and 2002 ($n=40$).

Independent variable	Observation time	2001	2002
Terminal bud cessation	mid-Aug.	0.7620 ***	0.7578 ***
	mid-Sep.	0.8977 ***	0.7544 ***
Terminal bud stage	mid-Aug.	-----	0.7146 ***
	mid-Sep.	-----	0.8491 ***
Leaf drop percent	early-Nov.	0.4303 **	0.1924 ns
	late-Nov.	0.5448 ***	0.4272 **
Leaf number	Not applicable	-0.7263 ***	-0.6345 ***
Shoot length (cm)	Not applicable	-0.7278 ***	-0.7150 ***
Leaf no./shoot length ratio	Not applicable	0.6614 ***	0.5136 ***

ns, **, and *** not significant at $P = 0.05$, and significant at $P = 0.01$, 0.001 , respectively.

Leaf drop was greater with tip grafts in 2001, but in 2002, there was no significant difference. In 2002, leaf drop was still low in early November (Figure 3.3). In both years, crabapple scions had the earliest leaf drop within each grafting system. By contrast, in basal grafting, 'Ottawa 3' and 'Macintosh' consistently were the latest, and in tip grafting,

'Golden Delicious' and 'Macintosh' were the latest for leaf drop (Figure 3.3). Leaf drop was significantly correlated with winter survival in 2001, but the correlation in late November was higher than early November in 2001 (Table 3.2). In 2002, only late November leaf drop was correlated with winter survival.

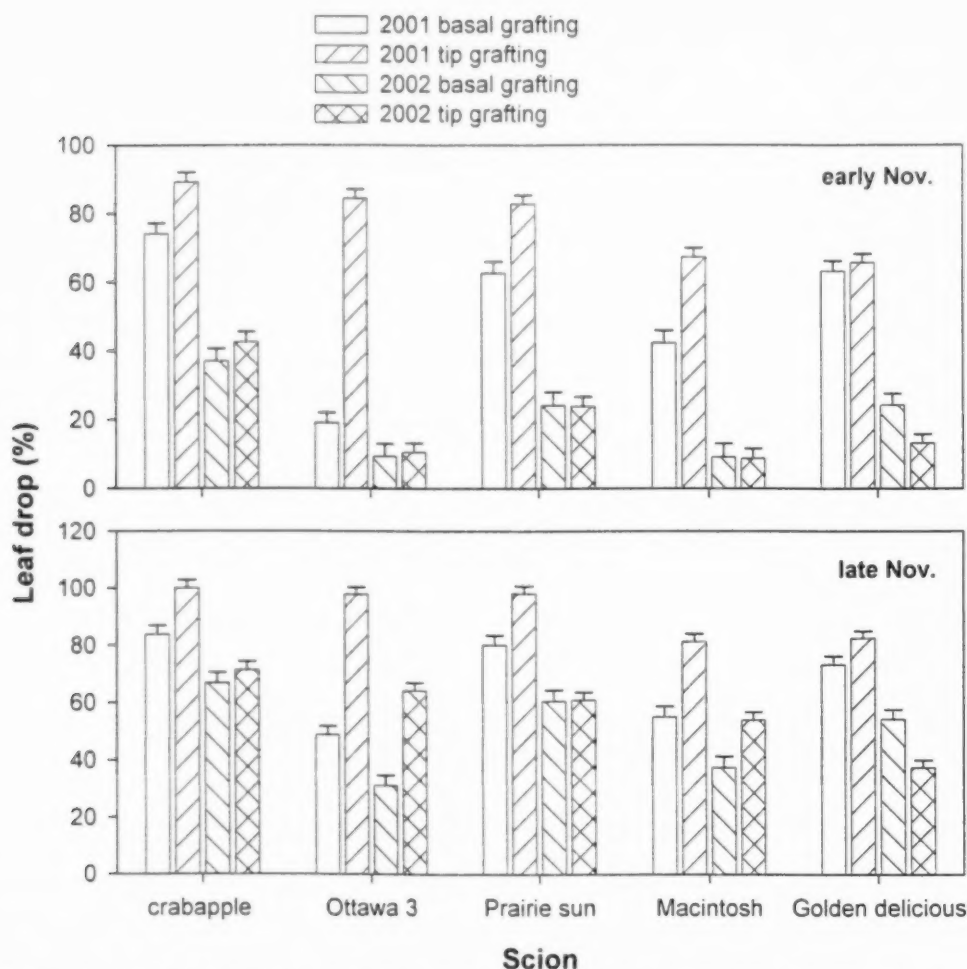


Figure 3.3 Leaf drop (%) of apple grafts as affected by grafting system and scions in early and late November in 2001 and 2002. Vertical bars indicate SE.

c. Vegetative growth

Tip grafts had shorter shoot lengths by 15 cm compared with basal grafting and leaf numbers were lower by ten leaves. Among the basal grafts, 'Golden Delicious' and 'Macintosh' had the longest shoot lengths and highest leaf numbers (Table 3.3) but leaf numbers were not significantly different among tip grafts. Although tip grafting decreased both the shoot lengths and leaf numbers of apple scions, the mean leaf

number to shoot length ratio of tip grafts was 3.2 times greater than that of basal grafts (Table 3.3). There was no significant difference in leaf number to shoot length ratio among basal grafts. Among tip grafts however, 'Ottawa 3' and crabapple scions had the highest and lowest leaf number to shoot length ratio, respectively (Table 3.3). From the regression analysis, leaf number and shoot length was negatively and leaf number to shoot length ratio was positively correlated with winter survival in both years (Table 3.2).

Table 3.3 Apical shoot leaf number, length and leaf number to shoot length ratio of apple grafts as affected by grafting system and scions over two years.

Scion	Grafting system	Leaf no.	Shoot length	Leaf no./shoot length
Crabapple	Basal grating	15.3 d ^z	19.7 c	1.3 d
	Tip grafting	10.0 e	7.3 e	3.4 c
Ottawa3	Basal grating	18.8 bc	17.9 cd	1.5 d
	Tip grafting	10.0 e	4.0 fg	6.7 a
Prairie sun	Basal grating	17.4 cd	15.6 d	1.7 d
	Tip grafting	9.2 e	3.3 g	5.5 b
Macintosh	Basal grating	20.9 ab	25.8 b	1.2 d
	Tip grafting	8.5 e	3.6 fg	5.3 b
Golden delicious	Basal grating	23.6 a	35.3 a	0.8 d
	Tip grafting	9.7 e	5.6 ef	3.8 c

^x Mean separation by least square means multiple comparison procedure at P = 0.05. Numbers followed by different letters within a column were significantly different.

Discussion

a. Effects of Tip Grafting

This study indicated cold hardiness of the apple scions was improved intensively by tip grafting on crabapple tree stocks compared with basal grafting on 'Ottawa 3' rootstocks (Table 3.1). 'Golden Delicious' is a very cold sensitive cultivar. There is no report to date indicating this cultivar can survive in the prairie provinces of Canada. When tip-grafted onto crabapple tree stocks, it had about 76% winter survival during the observation period. However, when basal-grafted onto 'Ottawa 3' rootstocks, most died and the remaining ones had extensive winter kill. These results suggested that tip grafting could be a valuable method for maintaining non-hardy germplasm for use in breeding. It could be used to maintain hybrids that are the results of hardy parents crossed to non-hardy parents to introgress genes into hardy germplasm. Good fruit quality is much rarer than cold hardiness in the current breeding populations so it could

be beneficial to tip graft first and judge hardiness later. Winter survival of tip grafts was slightly correlated with that of basal grafts ($r = 0.5260$, $n = 40$, $P = 0.0005$), which indicated that selection for cold hardiness cannot be done precisely on tip grafted trees, thus additional screening or a modification of the system developed would need to occur.

The results suggests that cold hardiness could be graft transmitted from rootstocks to scions. The less cold hardy 'Ottawa 3' scions grafted onto the cold hardy crabapple rootstocks showed increased winter survival. On the other hand, the cold hardy crabapple scions grafted onto the less cold hardy 'Ottawa 3' rootstocks showed decreased winter survival.

'Ottawa 3' is a dwarf rootstock while crabapple is a vigorous rootstock. In this experiment, autografts of 'Ottawa 3' had larger leaf numbers and longer shoot lengths compared with autografts of crabapple (Table 3.3). Patterson (1936) and Blair (1952) indicated that cold hardiness of cold sensitive standard apple varieties can be improved by top working rather than by basal grafting onto cold hardy rootstocks, which suggested that rootstock genotypes had less improvement on cold hardiness of scions than physiological age. The age of the tree can modify the time of cessation of growth (Faust, 1989; Guak and Fuchigami, 2001). Studies done on forest trees indicated older trees are cold hardier than juvenile ones (Li and Adams, 1993), because older trees tend to have earlier growth cessation in summer or early fall (Li and Adams, 1993; Lim et al., 1999). This earlier growth cessation would be related to the advanced development of cold acclimation.

b. Effects of cultivar scions

'Golden Delicious' originated from West Virginia; 'Macintosh' and 'Ottawa 3' from eastern Canada; 'SK Prairie Sun' from Saskatchewan, and crabapples were from Siberia but selected in the Canadian provinces. Cultivars from high latitudes are adapted to shorter growing seasons and prepare for dormancy early in the season and thus suffer less damage from freezing temperatures. As 'Golden Delicious' is a low-latitude cultivar it stopped vegetative growth late in the fall which contributed to its poor winter survival. 'Macintosh', as a less cold hardy cultivar, had the intermediate vegetative growth and terminal bud cessation and winter dieback. The results suggest that under field conditions, terminal bud cessation could be used to predict the initiation of cold acclimation at the cultivar level. Hurme et al., (1997) found that northern populations of Douglas fir set buds earlier than the southern ones in their native areas and when grown in the same environment, indicating genetic differences in the timing of bud set between populations.

c. Physiological mechanisms of cold hardiness affected by tip grafting

The close correlation between terminal bud cessation and winter survival indicated that tip grafting improved cold hardiness of scions by inducing early growth

cessation and cold acclimation of apple scions. Among basal grafts, cold hardy scions had early and cold sensitive scions had late terminal bud cessation. Reduced vegetative growth could allow more energy to be diverted into storage compounds and preserve more assimilates in perennial woody tissues. Other studies showed that development of cold hardiness started during the terminal bud formation period and was accelerated when most of the terminal buds had formed (Hurme et al., 1997). In this study, terminal bud cessation evaluated in mid-August and mid-September was closely related to winter survival. The first frost usually occurred in early September in our area, so terminal bud cessation around the first frost may be most useful for predicting winter survival.

Leaf drop was slightly correlated with cold hardiness if leaf drop was observed in late November (Table 3.2). Since the transition from paradormancy to endodormancy occurs during leaf fall, the time of leaf drop had been used as an indicator of cold hardiness (Chanasyk, 1953; Adrichem, 1970; Wood and Reilly, 2001). Leaf drop in raspberries determined by measuring the part of the cane from which the leaves had abscised and calculated as a percent of the total cane length was closely associated with winter hardiness (Adrichem, 1970). Measuring when half of the leaves abscised in the upper third of canes, Zatylny et al. (1996) found leaf drop was not correlated with field winter survival. The difference between these experiments may have resulted from the time and methods of evaluation. The time for 50% leaf drop in the upper 1/3 raspberry cane may be too short. In 2002, leaf drop was not related to winter survival when observed in early November, as leaf drop was still low then (Figure 3.3). Leaf drop was useful for predicting winter survival when observed in late November, after which cold acclimation might be completed and shoots are prepared for winter.

Conclusions

Tip grafting on crabapple tree stocks increased winter survival of apple scions compared to basal grafting on 'Ottawa 3' rootstocks. Cold hardiness could be graft transmitted from stocks to scions. Mature crabapple tree stocks were cold hardy, so they could be used to conserve less cold hardy parental materials and handle seedling populations in breeding programs. Basal grafting onto 'Ottawa 3' enhances the difference between varieties and may be a valuable screening tool for predicting cold hardiness in a breeding program.

Tip grafting enhanced terminal bud cessation and induced early leaf drop of scions relative to basal grafting. This resulted in earlier onset of cold acclimation and enhanced winter survival. The reduced vegetative growth in the tip grafting system could save more energy to be diverted into storage compounds and reserve more assimilates in perennial woody tissues, thereby improving tree quality and causing better cold acclimation. Terminal bud formation and winter survival had the highest correlation in this study. This indicated that under natural conditions, terminal bud cessation could be used to predict the initiation of cold acclimation and winter

4.0 Effect of tip grafting on early flowering and cold hardiness of seedlings of (*Prunus cerasus* × *P. fruticosa*) hybrids and *Amelanchier alnifolia*.

Introduction

To develop new fruit cultivars, tree fruit breeders need to evaluate fruit and tree characteristics as early as possible, but a long juvenile period has been a major impediment. Shortening the juvenile phase can shorten the selection cycle and thus decrease the costs of a breeding program. Burbank's success with tip grafting cherry seedlings onto a mature tree presents a method which may promote early transition from the juvenile to the adult phase, but his study did not include seedling age or proper controls. In Saskatchewan, the effect of low temperature on tip grafting and on the cold hardiness of the scions must also be determined.

Vegetative growth is closely related to reproductive development (Burbank and Faust, 1982) and the vigor of vegetative growth as measured by trunk diameter is positively correlated with the precocity of apple and pear seedling (Visser, 1964). Vegetative growth that is too vigorous or too weak can inhibit flower bud differentiation.

Cessation of growth is a prerequisite for cold acclimation in woody perennials and maximum hardiness occurs after leaf abscission (Weiser, 1970; Fuchigami et al., 1971; Arora et al., 1992). Consequently, terminal bud formation and leaf retention could be used as indicators of cold acclimation and cold hardiness (Proebsting, 1978; Guak, and Fuchigami, 2001; Wood and Reilly, 2001).

The objectives of this study were to determine the effect of tip grafting on flowering and cold hardiness and on the physiological mechanisms of cold hardiness and phase change. In this study, tip grafting of sour cherry hybrids and Saskatoon seedlings was conducted. The effects of mature tree stocks on flowering, winter dieback, terminal bud formation, leaf retention, and vegetative growth of tip-grafts were evaluated.

Materials and methods

Sour cherry seedlings ('SK Carmine Jewel' × 'Érdi bőtermő') were planted with a 0.33m spacing in rows, at the Horticulture Field Laboratory at University of Saskatchewan, Saskatoon in 1998 and used for scions in May 2000. 'SK Carmine Jewel' is a dwarf cultivar released at the University of Saskatchewan with 75% *P. cerasus* and 25% *P. fruticosa*. 'Érdi bőtermő' is a *P. cerasus* cultivar from Hungary. Three buds from 29 seedlings were tip grafted onto each of three 10-year-old bushes.

40 seedlings of Saskatoons, 'Nelson' open-pollinated, were grown in pots for two years tip grafted onto three 5-year-old mature trees (cv. 'Pembina'). Three buds from each seedling were individually chip budded onto the tip of the branches of the three mature trees.

Data were gathered in the fall 2001 and spring 2002, after two growing seasons. Tip grafts were compared to other branches on the stock trees (Stock Control) and the bushes that scions were taken from (Scion Control). Shoots in stock controls at similar height and length as tip grafts were measured.

Flowering or lack of it, was noted when the orchard was in full bloom. Shoot length and diameter were measured in November after leaf fall. Shoot length was measured from the grafted bud scale scar to the shoot tip and shoot diameter was measured at the grafted bud scale scar. Winter dieback was measured as a percentage of shoot length and as a percentage of shoots having any dieback. Terminal bud formation was assessed on apical shoots mid-September on sour cherries and in mid-July on Saskatoons by the following scale:

- 0 = not formed;
- 1 = apical meristem visually free;
- 2 = half green bud protrude, the terminal two leaves equal;
- 3 = whole bud protrude and tip start to turn brown;
- 4 = half turn brown;
- 5 = totally formed.

Total leaf number was counted in September after terminal buds formed. Leaf retention was measured in late October.

SAS program (SAS institute, 1999) was used for statistical analysis. All binomial data, i.e. winter dieback and flowering, were analyzed using GENMOD procedure. Other data, i.e. vegetative growth, terminal bud formation and leaf drop was analyzed using GLM procedure. Before analysis, leaf drop percent was subjected to an arc sine square root transformation.

Results

a. Flowering and winter dieback

Tip grafts decreased flowering in sour cherry seedlings, but enhanced flowering in saskatoon seedlings compared to controls (Table 4.1). No winter dieback symptoms were seen in tree-stock controls. Tip-grafts had less winter dieback than scion controls in cherries but had little effect on cold hardiness in Saskatoons (Table 4.1).

Table 4.1 Flowering and winter dieback of tip grafts compared to other branches on the stock trees (tree-stock control) and the bushes that scions were taken from (Scion Control). Numbers followed by different letters within a column were significantly different at $P = 0.05$.

Source	Sour cherry				Saskatoon		
	Shoot with flowers (%)	Flower no.	Shoot dieback (% of length)	Shoots with dieback (%)	Shoot with flowering (%)	Flower no.	Shoots with dieback (%)
Tree-stock control	100 a	2.5 a	0.0 b	0.0 b	100 a	6.3 a	0.0ns
Tip grafts	9.8 c	0.2 c	0.5 b	4.9 b	71.0 b	10.2 a	12.4ns
Scion Control	42.3 b	1.4 b	3.4 a	69.2 a	22.6 c	2.0 b	12.9ns

b. Terminal bud formation and leaf drop

Tip grafting enhanced terminal bud formation on sour cherry seedlings (Table 4.2). The self-rooted sour cherry seedlings continued growing into September, while the terminal buds on the mature tree stocks and tip-grafted scions had formed by mid-August. Data collected in mid-September indicated that the terminal buds of mature tree stocks and tip-grafts were brown, but those of scion controls were still green. Tip grafting delayed terminal bud formation in Saskatoon seedlings, but it was not significantly different among the three sources of shoots. The terminal buds of the shoots from the three sources had formed by the end of July (Table 4.2).

Tip grafting enhanced leaf drop on sour cherry and saskatoon seedlings. At the end of October, leaves on the tree-stock control had almost completely dropped, but leaf drop on scion controls was only 47.8% and while on tip-grafts it was 78.7% (Table 4.2). The same trend was found on Saskatoons (Table 4.2).

Table 4.2 Terminal bud formation and leaf drop of tree-stock controls, tip grafts and scion controls of sour cherry and Saskatoons. Numbers followed by different letters within a column were significantly different at $P = 0.05$.

Source	Sour cherry		Saskatoons	
	Terminal bud formation	Leaf drop (%)	Terminal bud formation	Leaf drop (%)
Tree-stock control	5.0 a	96.6 a	4.0 ns	100 a
Tip-grafts	4.7 a	78.7 b	3.8 ns	42.9 b
Scion Control	2.7 b	47.8 c	4.2 ns	31.4 c

c. Vegetative growth

The influence of mature trees on the vegetative growth of tip-grafts was significant as their growth approximated the mature trees (Table 4.3). In sour cherries tip grafts had an 84% decrease in shoot length and 76% decrease in shoot diameter relative to the scion controls (Table 4.3). In Saskatoons, tip grafting increased shoot length by 257% and shoot diameter by 42% compared with scion controls (Table 4.3).

Table 4.3 Vegetative growth of tree-stock controls, tip grafts and scion controls of sour cherry and Saskatoons. Numbers followed by different letters within a column were significantly different at $P = 0.05$.

Source	Sour cherry		Saskatoons	
	Shoot length (cm)	Shoot diameter (mm)	Shoot length (cm)	Shoot diameter (mm)
Tree-stock control	13.1 b	2.48 b	30.2 a	4.02 b
Tip-grafts	12.4 b	3.01 b	34.7 a	4.62 a
Scion Control	101.6 a	12.53 a	9.7 b	3.26 c

Discussion

a. Effect of tip grafting on flowering

All the tree-stock controls of both species set flowers. Tip grafts of sour cherries had less flowers than scion controls, while tip grafts of Saskatoons had more flowers than scion controls (Table 4.1). Vegetative growth of tip grafts of both species was similar the to trees on which they were grafted. The results from this study indicated that tip grafting reduced vegetative growth and restricted the flower bud induction in sour cherry seedlings, while it improved vegetative growth and flower bud induction in Saskatoons seedlings. The effect of tip grafting on flowering may be not through graft transmission of mature status but rather though graft transmission of vigor from the

rootstock to scions. Visser (1964) concluded that there was a significant negative correlation between the juvenile period and the vigor of the seedling as measured by trunk diameter. A similar cases of rootstocks inflencing vigour and flowering has been seen in several species (Visser, 1964; 1965; Visser et al., 1976; Zimmerman et al., 1970; 1971; 1977). Zimmerman (1971; 1972b) stated that growing apple seedlings under optimum growing conditions in the greenhouse, which produced the most vigorous growth of the seedlings, had the greatest effect on shortening the juvenile period. Aldwinckle (1975) manipulated plants for early flowering by promoting vegetative growth of the apple seedlings. Conversely, methods used by horticulturists routinely to slow growth, such as girdling, scoring, bark inversion, root pruning, training the branches in a horizontal position, applying growth retardants, and using dwarfing rootstock, actually increase the length of the juvenile period (Zimmerman, 1972; Hackett, 1985).

Flower bud initiation in both sour cherries and Saskatoons in this study was not related to terminal bud formation. In sour cherries, terminal bud formation of tip-grafts was higher than that of scion controls, but tip-grafts had less percent flowering. In Saskatoons, terminal bud formation of tip-grafts was lower than that of scion controls, but tip-grafts had more flowers. This result concurs with that obtained by Steeves and Steeves (1990) but is contrary to what reported by Faust and Buban (1985). Steeves and Steeves (1990) found that inflorescence initiation in *A. alnifolia* was not correlated with shoot growth cessation, while Faust and Buban (1985) stated that flower bud initiation started only after terminal bud formation in apple trees. My study indicates that the mechanism of flower bud initiation is not controlled by terminal bud formation for the first flowering young seedlings. In sour cherries, the axillary buds can be induced to flower buds and the terminal buds are only leaf buds. Therefore, the terminal bud formation does not affect the flower bud initiation in axillary buds. In Saskatoons, the fact that inflorescence initiation is not correlated with terminal bud formation may be due to the very early terminal bud cessation compared with apple trees.

In this study, tip grafting did not enhance flowering in sour cherry but did enhance flowering in Saskatoons compared to the scion controls. In this study, taking three buds away from the pot-grown Saskatoon seedlings could weaken the plants. If we can use bigger pots, the vegetative growth may be improved and the juvenile period may be shortened without tip grafting. Way (1971) demonstrated that grafting of apple seedlings, which have a relatively short juvenile period, onto tree-stock control induced precocity. He also pointed out that it was not economically feasible due to expensive labor and scion loss from branch competition or virus infection. Sour cherry hybrids and Saskatoons are small fruit trees. The juvenile periods are usually only 3-5 years, so tip grafting of these two species maybe not practical.

b. Effect of tip grafting on cold hardiness

Tip grafting on sour cherries decreased winter dieback dramatically (Table 4.1). The effect of tip grafting on enhancing cold hardiness might be due to its effect on terminal bud formation and leaf drop. Tip grafting increased terminal bud formation and leaf drop. The most severe low temperature injury to fruit trees usually occurs in late fall

or early winter and under such conditions cold acclimation is of prime importance (Westwood, 1970).

In Saskatoons, tip grafting had little influence on winter dieback. Although tip grafts had lower terminal bud formation compared to scion controls, terminal buds of tip-grafts had formed by early July (Table 4.2). Kaurin et al (1984) found that the time of onset of vegetative maturity in 'Smoky' occurred very early in summer on 29 May and the initiation of cold acclimation was correlated with the cessation of growth. This early onset of terminal bud formation of Saskatoons may results in the adaptation to cold environmental stress. Cold hardiness is developed from cold acclimation, which starts after growth cessation. In Saskatoons, there is enough time for cold acclimation development before harsh winter in shoots from the three sources: non-grafted mature trees, tip-grafts, and scion controls, so there is no difference on winter dieback among the three sources.

Leaf drop was earlier in tip-grafts than in scion controls in both sour cherries and Saskatoons (Table 4.2). This early leaf drop may be due to the reduced vegetative growth by the tree-stock control (Table 4.3). The results in this study were in agreement with Guak and Fuchigami (2001), who reported that the rate of leaf abscission was inversely proportional to tree vigor.

Conclusions

In sour cherries, tip grafting reduced the vegetative growth and enhanced cessation of shoot growth compared with scion controls. The shoots will be delayed to attain the minimum size for flower initiation, and subsequently, floral bud development will be retarded. Earlier growth cessation leads to an earlier onset of dormancy and decreases the risk of cold injury, so additional procedure should be conducted for cold hardiness screening of seedlings. Tip grafting on Saskatoons increased the vegetative growth compared with scion controls and enhanced flower bud induction. The increased vegetative growth resulted in not only higher percent of flowering seedlings but also higher flower cluster number. So tip grafting on Saskatoons may provide one year earlier for fruit quality evaluation than scion controls. Due to the relatively short juvenile period and increased cold hardiness by mature tree stocks, tip grafting of sour cherry and Saskatoon seedlings is likely not beneficial to tree fruit breeders, unless it is used to increase survival of non-hardy germplasm..

5.0 Juvenility of scions from various maturity zones of apple seedlings

Introduction

In apple breeding programs, apple seedlings start to set fruit only after an extended period, which may last 7 to 12 years. This non-flowering period after seed germination is called juvenile period. Attainment of the ability to flower indicates the end of the juvenile phase and attainment of the adult conditions. This transition from the juvenile through transitional to the mature phase has been referred to as phase change. Associated with

this phase change are morphological and physiological changes, such as branch number, shoot growth vigor, ability to form adventitious roots and buds, seasonal leaf retention, and spring bud break. When lacking reproductive structures, juvenile and adult phases may be distinguishable by leaf phyllotaxy, shape, size, and color; stem branching and tropism; thorniness; and other vegetative characteristics (Huang et al., 1992).

Alterations in morphology and anatomy associated with phase change have been used as markers of maturity or juvenility in woody plants (Rogler and Hackett, 1975a). Since the morphological changes are progressive, they can even be used as indicators of the relative level of physiological maturity of plant parts (Brand and Leneberger, 1992).

Observations made on cold hardiness of the two groups of trees propagated on 1- and 2-year-old Jonathan seedling rootstock shown that trees propagated from the juvenile zone of certain seedlings were slightly hardier than those from adult zone of the same seedlings (Lapins, 1962). This result was contrary to that obtained by Lim et al. (1999), who found that freezing tolerance increased with both chronological age and developmental phase.

The objectives of this study was to determine the relative cold hardiness of the juvenile and adult zones of apple seedlings; to determine both the vegetative and reproductive response of tip-grafted juvenile and mature scions onto mature rootstocks; and to find the parent physiological indicators for phase change.

Materials and methods

Four genotypes of apple selections were used for being taken scions from six different heights from the ground. The propagation materials representing the various maturity levels were taken from below 0.25m, 0.5m, 1.0m, 1.5m, and 2.0m above the ground and adult zone. Two mature 'Selin' crabapple trees were chosen to receive tip grafting. Each tree held four replications. Buds taken from the current year's shoots on the seedlings were chip-budded onto the periphery of the 'Selin' apple trees. A randomized complete block design with four replicates, four buds with each replicate for a total of 384 buds.

The vegetative growth was assessed on shoot length and shoot diameter. Shoot length was measured between the graft union bud scale scar and the tip of the shoot. Shoot diameter was measured at the bud scale scar. Flowering was evaluated by the percent shoots with flowers in spring 2002 and 2003.

Winter damage was determined by the percentage of shoot dieback after winter in the following spring. Because no apparent shoot dieback in spring 2002, winter damage was not assessed. Terminal bud formation was assessed in mid-August by dividing the terminal buds into six levels: 0=not formed; 1= apical meristem visually free; 2= half green bud protrude, the terminal two leaves equal; 3 = whole bud protrude and tip start to turn brown; 4 = half turn brown; 5 =totally formed. Leaf retention was evaluated in mid-October by the percentage of total leaf retained on the shoots.

Procedure GLM was used for an analysis of variance using RCBD model (SAS institute, 1999). When significantly different, means were separated using least square means multiple comparison at $P=0.05$.

Results

a. Flowering

Adult shoots had the highest flower number. No flowers were formed on juvenile tip grafts and tip grafts derived from 0.5m zone (Figure 5.1). After two growing seasons in spring 2003, flowering was not consistent with that in spring 2002, tip grafts from scions 2.0m had higher flowering percent than those from adult scions.

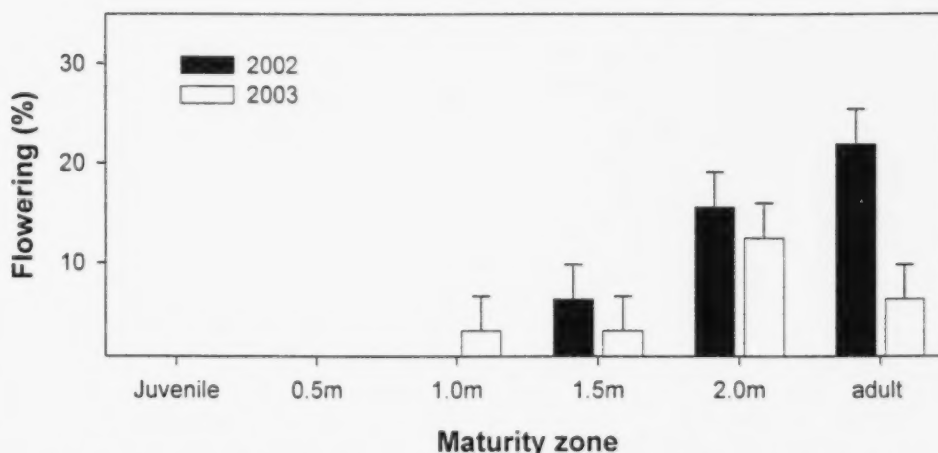


Figure 5.1 Flowering of apple tip grafts onto 'Selin' crabapple trees as affected by the maturity zones of apple seedlings in spring 2002 and 2003, one and two growing seasons after grafting, respectively.

b. Terminal bud development and leaf drop

Terminal bud development was not affected by genotypes and maturity zones. There was no interaction between genotypes and maturity zones. Terminal bud development was significantly affected by years, with further development in 2001 than in 2002. In both years, terminal buds formed earlier on adult tip grafts and later on juvenile shoots. Means of terminal bud formation showed that adult tip grafts had earlier terminal bud formation than those from scions below 2.0m above ground (Figure 5.2).

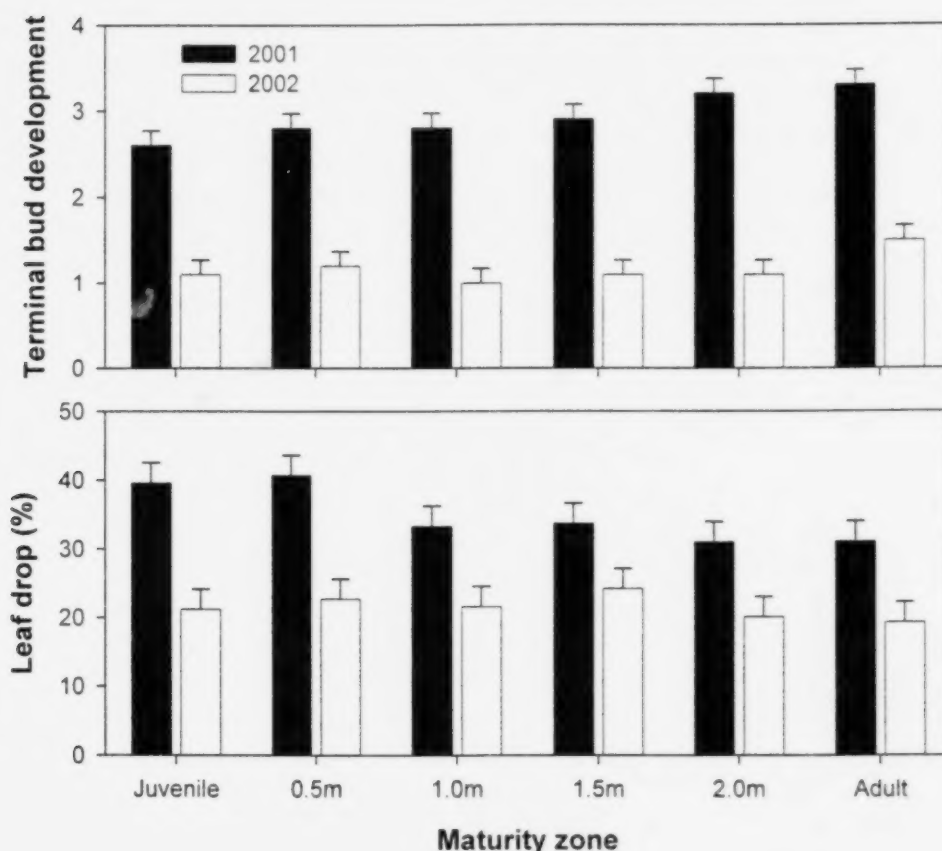


Figure 5.2 Terminal bud development and leaf drop of apple tip grafts onto 'Selin' crabapple trees as affected by the maturity zones of apple seedlings in 2001 and 2002, one and two growing seasons after grafting, respectively.

Leaf drop was not affected by maturity zones but significantly affected by genotypes. Leaf drop in 2002 occurred much later than that in 2001 and the rate varied in different years, but the leaf drop trend was the same among genotypes between two years (Figure 5.3).

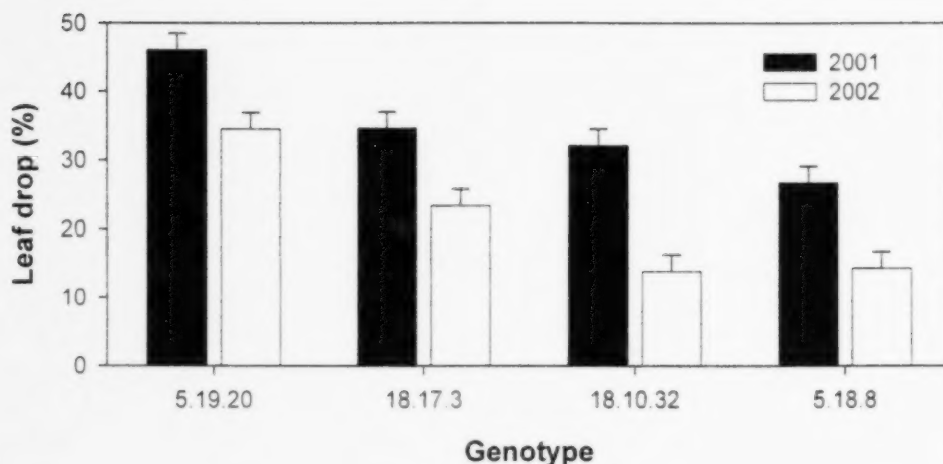


Figure 5.3 Leaf drop of apple tip grafts onto 'Selin' crabapple trees as affected by the genotypes of apple seedlings in 2001 and 2002, one and two growing seasons after grafting, respectively.

c. Vegetative growth

Vegetative growth including shoot length and shoot diameter was not significantly affected by year. Shoot length was affected by genotypes and maturity zones, but there was no interaction between genotypes and maturity zones on shoot length. Shoot diameter was only affected by genotypes, and there was no interaction between genotypes and maturity zones. Shoot length was higher on juvenile shoots, and decreased with maturity, being the lowest on adult shoots in both years (Figure 5.4).

After two growing seasons after grafting, thorns and lateral shoots were developed on the grafts. Thorn number and lateral shoot number was affected by maturity zones but not affected by genotypes. There was no interaction between maturity zones and genotypes on thorn number and lateral shoot number. Both thorn number and lateral shoot number were the highest on juvenile shoots and the lowest on adult shoots (Figure 5.5).

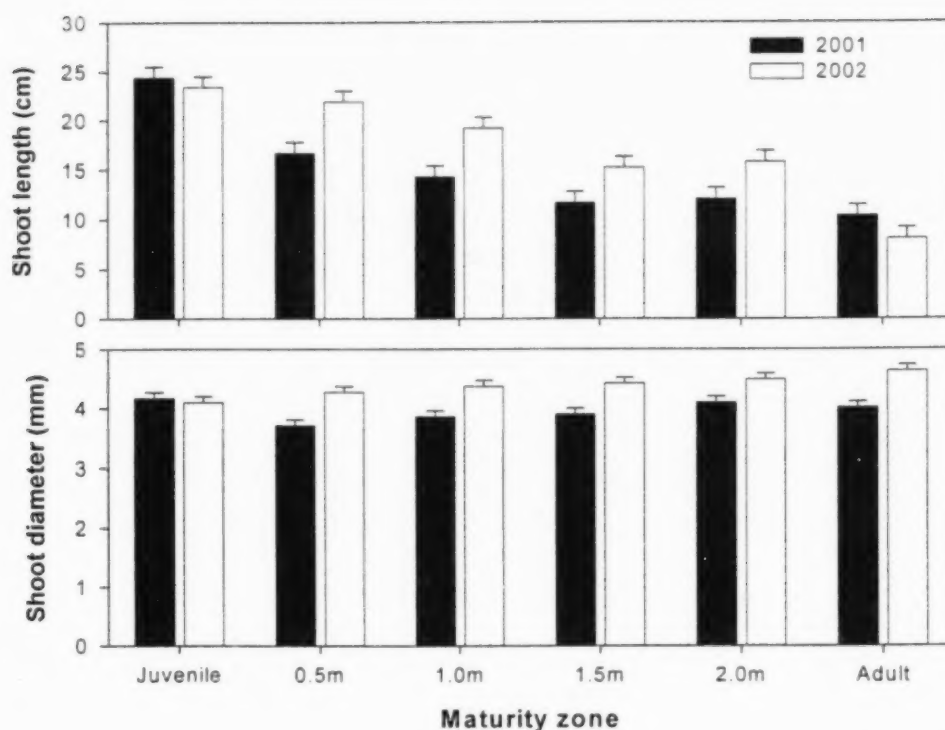


Figure 5.4 Shoot length and shoot diameter of apple tip grafts onto 'Selin' crabapple trees as affected by the maturity zone of apple seedlings in 2001 and 2002, one and two growing seasons after grafting, respectively.

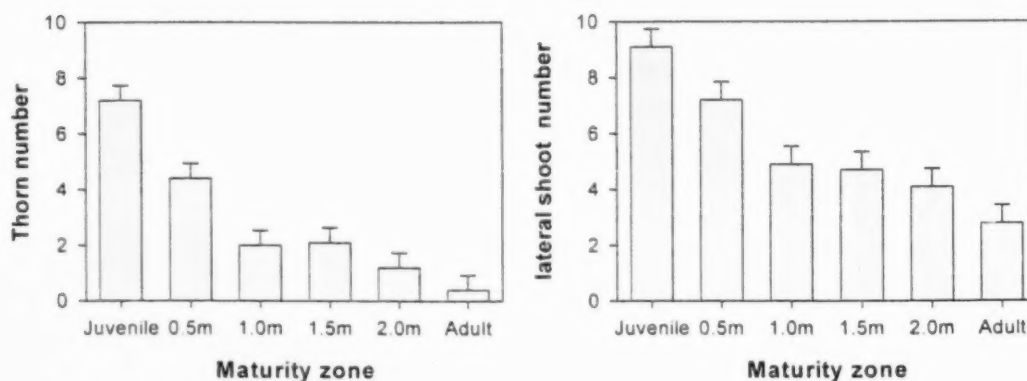


Figure 5.5 Thorn number and lateral shoot number of apple tip grafts onto 'Selin' crabapple trees as affected by the maturity zone of apple seedlings in 2002, two growing seasons after grafting.

Discussion

The shorter length of adult shoots grew fewer leaves but thicker stems because energy would have been diverted from radical growth to diameter growth. The leaf number to shoot length ratio was higher in adult tip grafts. Compared with juvenile tip grafts, adult tip grafts had higher leaf area per shoot length, so the adult tip grafts could assimilate more photosynthates into the shoots. Adult tip grafts had thicker shoot diameter, and more organic chemicals can be preserved in phloem. The shoots from adult zone also had higher leaf retention and fewer branches than that from juvenile zone, so perhaps shoots from adult zones could concentrate more assimilates to the buds for flower bud formation. Lim et al. (1999) found that freezing tolerance increased with developmental phase change by comparing mature and juvenile cuttings. The nutritional conditions, especially carbohydrates, such as sucrose, are also the necessities for cold hardiness. So it is reasonable that adult tip grafts had better freeze resistance.

The most important factors that control the transition from the juvenile to the mature conditions are size and node number of the plant rather than the chronological age or number of growing cycles (Hackett, 1983). The results in this study showed that adult tip grafts had less leaf number and shorter shoot length than juvenile tip grafts, and leaf number and shoot length were linearly associated with maturity, which indicated that a minimum size and node number must be obtained for transition to the mature conditions (Zimmerman, 1971).

The apple selections had set fruit, indicating winter survivability, and there was no apparent difference on winter survival in either years. However, terminal bud formation were significantly different between adult and juvenile tip grafts, and was negatively related to maturity in both data collecting years. Observations made on cold hardiness of the two groups of trees propagated on 1- and 2-year-old 'Jonathan' seedling rootstock shown that trees propagated from the juvenile zone of certain seedlings were slightly hardier than those from adult zone of the same seedlings (Lapins, 1962). This result was contrary to that obtained by Lim et al. (1999). By comparing mature ortets with juvenile cuttings, they found that freezing tolerance increased with both chronological age and developmental phase change.

Juvenile characteristics, such as rooting potential, may be preserved at the base of plants in ontogenetically young tissues while maturation occurs in the periphery of the plants in ontogenetically older tissues (Zimmerman, 1971). In fall, leaves in the base of a poplar tree retained longer than those on the top of the tree (Personal observation). Although one of the juvenile characteristics is high leaf retention in fall (Janick et al., 1996), leaf retention in this study was not closely related to juvenility or maturity, and on the contrary, leaf retention was a bit higher on adult tip grafts. Leaf drop in raspberries determined by measuring the part of the cane from which the leaves had abscised and calculated as a percent of the total cane length was clearly associated with winter hardiness (Adrichem, 1970). Leaf retention based on the percentage of leaflets retained was used as atypical symptom of cold damage to pecan (Wood and Reilly, 2001). However, results are not consistent. The time of cane leaf drop in raspberries recorded as days from Sept. 1 until half of the leaves in the upper third of the cane had abscised was not correlated with field survival (Zatylny et al., 1996). In this study, leaf retention

was not correlated with maturity, and there was no significant difference on leaf retention among different maturity levels. This may due to the small difference of cold hardiness among the maturity levels from the same genotype and the cold hardy genotypes chosen have survived through to flower, or due to increased cold hardiness by tip grafting onto mature trees. Leaf drop in 2001 occurred sooner than in 2002 and the rate varied in different years, but the relative amounts between genotypes were the same for each year (Adrichem, 1970).

In this study, the average juvenile period of the self-rooted apple selection seedlings is about 11 years. When the mature scions were taken from the adult zone of the seedling and grafted, they started to set fruit one to three years after grafting. So, the time needed for an adult scion to set fruit is juvenile period plus vegetative period, which is at least 11 years.

No flowers were formed on tip grafts of scions from below 1.0m zones, but flowers were formed on tip grafts of scions from above 1.0m zone even after one growing season after grafting (Figure 5.1). Overall considering of all the parameters related to the maturity, we could conclude that the transition zone might be between 1.0 and 2.0m. This transition zone is lower than that concluded by Zimmerman (1971), which indicated that tip grafting might decrease the juvenility and enhance the transition from juvenile to adult phase. However, tip grafting may be more beneficial to shortening juvenile period when it is done after the seedlings have been more than 1.0m tall and the scions are taken from above 1m from the ground.

By working on apple and pear scion controls, Visser (1976) showed that the inheritance of the juvenile period is additive in nature, a mode of inheritance that is a function of multi-genetic factors governing development. There is a high correlation between the length of the vegetative phase (time from vegetative propagation to fruiting) of a cultivar and the length of the juvenile period of its progeny (Visser, 1965). The correlation between the length of the vegetative phase of a tip graft created from this study and the maturity level were high. The less mature, the longer the juvenile period, and the longer the vegetative phase. Therefore, this study can extend Visser's statement from another way: the length of the vegetative period of a selection is highly correlated with the juvenile period of its mother seedling.

There were significant differences between tip grafts from juvenile zone and adult zone of the apple seedlings on vegetative growth and terminal bud formation. Vegetative growth was closely related to reproductive growth, and the morphological characteristics were repeatable for using as morphological markers for phase change. The high degree of significance of correlation between morphological characteristics and maturity in the present study clearly demonstrates the progressive phase change. The transition of the buds from juvenile to the adult phase occurred at a height of 1.0-2.0 m, which was lower than that reported on self-rooted apple seedlings, which indicated that tip grafting might decrease the juvenility and enhance the transition from juvenile to adult phase. Flowers formed on the tip grafts derived from the scions collected above 1.0m on the seedlings. These results suggested that tip grafting might be more beneficial to shortening juvenile period if buds are taken at the proper stage, such as when it is done after the seedlings have been more than 1.0m tall and the scions are taken from above 1m from the ground.

Conclusion

From juvenile zone to adult zone, there was a descendant tendency on leaf number, shoot length, thorn number and branch number, but an ascendant tendency on ratio of leaf number to shoot length, diameter, terminal bud formation and leaf retention. The shorter length of adult shoots grew fewer leaves, but higher ratio of leaf number to shoot length and thicker stems, so more energy would have been accumulated from flower bud initiation.

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GENERAL CONCLUSIONS

Tip grafting of sour cherry and Saskatoon seedlings, which have relatively short juvenile stages, is not economically feasible due to the expensive labor cost and scion loss because of branch competition or abiotic or biotic stress. However, it may be beneficial for apple seedlings which have a longer juvenile period. Since tip grafting also increases cold hardiness, and there is only a small correlation between the cold hardiness of tip grafts and basal grafts, care should be taken with tip grafting. When tip grafting is used to conserve breeding materials, basal grafting should also be done to test the relative cold hardiness.

When compared to basal grafting, tip grafting reduced the vegetative growth and enhanced terminal bud formation. Tip grafting also reduced the vegetative growth compared to well-grown scion controls. Vegetative growth was closely related to terminal bud formation. Earlier terminal bud cessation resulted in reduced vegetative growth, thereby leading to the earlier onset of cold acclimation and thereby increasing winter survival. Terminal bud formation and vegetative growth characteristics including leaf number, shoot length, the ratio of leaf number to shoot length can be used as physiological markers for early selection of precocity and cold hardiness.

The greatest benefit to using tip grafting in northern climates may be to enhance survival of less cold hardy varieties and hybrids and thus assist in the introgression of valued traits into adapted germplasm.

PROJECT DEVELOPMENT MATERIALS

A number of scientific results from our work have been presented at professional conference and published in professional proceedings and submitted in professional journal. A list of current and pending publications are provided below:

THESIS

Tip grafting for evaluation of cold hardiness and early flowering in tree fruit breeding program.

REFEREED PUBLICATIONS IN FINAL DRAFT FORMAT

Lu, Q and R.H. Bors. Comparison of self-rooted and tip-grafted seedlings of (*Prunus cerasus* × *P. fruticosa*) hybrids and *Amelanchier alnifolia*. (Manuscript submitted for review in August, 2002 to Acta Horticulture).

NON-REFEREED PUBLICATION

Lu, Q and R.H. Bors. Influence of tip grafting on juvenility of scions from various zones of apple seedlings. (PowerPoint presentation in 18th Annual Plant Sciences Graduate Student Symposium, Saskatoon, Saskatchewan, March 9, 2002)

Lu, Q and R.H. Bors. Comparison of self-rooted and tip-grafted seedlings of (*Prunus cerasus* × *P. fruticosa*) hybrids and *Amelanchier alnifolia*. (Poster presentation in XXIV International Horticultural Congress, Toronto, August 11-17, 2002) (Attached)

PUBLISHED ABSTRACTS

Lu, Q and R.H. Bors. Comparison of self-rooted and tip-grafted seedlings of (*Prunus cerasus* × *P. fruticosa*) hybrids and *Amelanchier alnifolia*. XXIV International Horticultural Congress, Toronto, August 11-17, 2002)

REPORTS

Bob Bors and Qiuju Lu, 2002. Investigation of tip grafting for evaluation of cold hardiness and juvenility in tree fruit breeding. Progress Report - December, for Saskatchewan Agriculture and Food, Agriculture Development Fund.

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OTHER OUTPUT FROM THE PROJECT

Grafting workshop, twice a year in July and August in U of Saskatchewan, Dept. of Plant Sciences.

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Note: ADF was acknowledged on the thesis, poster, presentation, reports and submitted papers.

REFERENCES:

- Adrichem, M.C.J.V., 1970. Assessment of winter hardiness in red raspberries. *Can. J. Plant Sci.* 50: 181-187.
- Aldwinckle, H.S., 1975. Flowering of apple seedlings 16-20 months after germination. *HortScience* 10: 124-126.
- Arora, R., M.E. Wisniewski, and R. Scorza, 1992. Cold acclimation in genetically related (sibling) deciduous and evergreen peach (*Prunus persica* Batsch). I. seasonal changes in cold hardiness and polypeptides of bark and xylem tissues. *Plant Physiol.* 99: 1562-1568.
- Blair, D.S., 1952. Trends in fruit research. p:9-12. In: Report of proceedings of the western Canadian society for horticulture, eighth annual meeting, February 18-20. Lethbridge, Alberta.
- Bors, R.H., 2003. Domestic fruit development program. ADF final report.
- Brand, M. H. and R.D. Lineberger, 1992. *In vitro* rejuvenation of betula (*Betulaceae*): biochemical evaluation. *Amer. J. Botany* 79: 626-635.
- Brown, A.G., 1975. Apple. p: 3-37. In: Janick, J. and J. N. Moore (eds.). *Advances in fruit breeding*. Purdue Univ. Press, W. Laffayette.
- Buban, T. and M. Faust, 1982. Flower bud induction in apple trees: internal control and differentiation. *Hort. Reviews* 4: 174-197.
- Burbank, L., 1921. Grafting and budding, Vol. 2. P. F. Collier & Son Company, New York.
- Chanasyk, V., 1953. Practical tests for hardiness. P: 63-66. In: Report of proceedings of the western Canadian society for horticulture, ninth annual meeting, February 16-18. Winnipeg, Manitoba.
- Dole, J.M. and H.F. Wilkins, 1992. In vivo characterization of a graft-transmissible, free-branching agent in poinsettia. *J. Amer. Soc. Hort. Sci.* 117: 972-975.
- Faust, M., 1989. Resistance of fruit trees to cold. p: 307-331. In: *Physiology of temperate zone fruit trees*. John Wiley & Son, New York.
- Fuchigami, L.H., C.J. Weiser, and D.R. Evert, 1971. Induction of cold acclimation in *Cornus stolonifera* Michx. *Plant Physiol.* 47: 98-103.
- Golecki, B. A. Schulz, U. Caustens-Behrens, and R. Kollmann, 1998. Evidence for graft transmission of structural phloem proteins or their precursors in heterografts of cucurbitaceae. *Planta* 206: 630-640.
- Guak, S. and L.H. Fuchigami, 2001. Effects of applied ABA on growth cessation, bud dormancy, cold acclimation, leaf senescence and N mobilization in apple nursery plants. *J. Hort. Sci. Biotech.* 76: 459-464.
- Hackett, W.P., 1983. Phase change and intra-clonal variability. *HortScience* 18: 840-844.
- Hackett, W.P., 1985. Juvenility, Maturation, and Rejuvenation in woody plants. *Hort. Reviews* 7: 109-147.
- Hansche, P.E., 1983. Plant size and number affect genetic analysis and the improvement of fruit and nut tree cultivars. *HortScience* 25: 389-393.
- Huang, L.-C, S. Lius, B.-L. Huang, T. Murashige, E.F.M. Mahdi, and R. van Gundy, 1992. Rejuvenation of *Sequoia sempervirens* by repeated grafting of shoot tips onto juvenile rootstock *in vitro*: Model for phase reversal of trees. *Plant Physiol.* 98: 166-173.
- Hurme, P., T. Repo, O. Savolainen, and T. Paakkonen, 1997. Climatic adaptation of bud set and frost hardiness in Scots pine (*Pinus sylvestris*). *Can. J. For. Res.* 27: 716-723.
- Janick, J., J.N. Cummins, S.K. Brown, and M. Hemmat, 1996. Apples p. 1-77. In: J. Janick and J.N. Moore (eds.) *Fruit breeding, VI. Tree and tropical fruits*. John Wiley & Sons, Inc., New York, USA.
- Kaurin, A., C. Stushnoff, and O. Junttila, 1984. Cold acclimation and dormancy of *Amelanchier alnifolia*. *J. Amer. Soc. Hort. Sci.* 109: 160-163.
- Lakso, A.N. 1994. Apple p: 3-42. In B. Schaffer and P.C. Andersen (eds.). *Handbook of environmental physiology of fruit crops. v1. Temperate crops*. CRC Press, Inc., Florida, USA.
- Lapins, K. 1962. Artificial freezing as a routine test of cold hardiness of young apple seedlings. *Proc. Amer. Soc. Hort. Sci.* 81: 26-34.
- Layne, R.E.C. 1994. *Prunus* rootstocks affect long-term orchard performance of 'Red haven' peach on Brookston clay loam. *HortScience* 29: 167-171.
- Layne, R.E.C. and P.Y. Jui, 1994. Genetically diverse peach seedling rootstocks affect long-term performance of 'Redhaven' peach on fox sand. *J. Amer. Soc. Hort. Sci.* 119:1303-1311.
- Li, P., and W.T. Adams, 1993. Genetic control of bud phenology in pole-size trees and seedlings of coastal Douglas-fir. *Can. J. For. Res.* 23: 1043-1051.

- Lim, C.C., S.L. Krebs, and R. Arora, 1999. A 25-kDa dehydrin associated with genotype- and age-dependent leaf freezing-tolerance in *Rhododendron*: a genetic marker for cold hardiness. *Theor. Appl. Genet.* 99: 912-920.
- O'Neil, G.A., A.N. Aitken, and W.T. Adams, 2000. Genetic selection for cold hardiness in coastal Douglas-fir seedlings and saplings. *Can. J. For. Res.* 30: 1799-1807.
- Okada, Y., A. Saito, M. Nishiguchi, T. Kinura, M. Mori, K. Hanada, J. Sakai, C. Miyazaki, and Y. Matsuda, 2001. Virus resistance in transgenic sweet potato [*Ipomoea batatas* L. (Lam)] expressing the coat protein gene of sweet potato feathery mottle virus. *Theor. Appl. Genet.* 103: 743-751.
- Palonen, P. and D. Buszard, 1997. Current state of cold hardiness research on fruit crops. *Can. J. Plant Sci.* 77: 399-420.
- Patterson, C.F., 1936. Hardy fruits. Published by the author, Saskatoon.
- Proebsting, E.L.J., 1978. Adapting cold hardiness concepts to deciduous fruit culture. p: 267-279. in P.H. Li and A. Sakai (eds.): *Plant cold hardiness and freezing stress. Mechanisms and crop implications.* Academic press, New York.
- Rinne, P.L.H., P.L.M. Kaikuranta, L.H.W. van der Plas, and C. van der Schoot, 1999. Dehydrins in cold-acclimated apices of birch (*Betula pubescens* Ehrh.): production, localization and potential role in rescuing enzyme function during dehydration. *Planta* 209: 377-388.
- Rogler, C.E. and W.P. Hackett, 1975. Phase change in *Hedera helix*: induction of the mature to juvenile phase change by gibberellin A3. *Physiol. Plant.* 34: 141-147.
- Sherman, W.B. and P.M. Lyrene, 1983. Handling seedling populations. p: 66-71. In: Moore, J. N. and J. Janick (eds.). *Methods in fruit breeding.* Purdue Univ. Press, W. Lafayette.
- Smirnov, S., Shulaev, V., and Tumer, N.E., 1997. Expression of pokeweed antiviral protein in transgenic plants induced virus resistance in grafted wild-type plants independently of salicylic acid accumulation and pathogenesis-related protein synthesis. *Plant Physiol.* 114: 1113-1121.
- Sonoda, S. and M. Nishiguchi, 2000. Graft transmission of post-transcriptional gene silencing: target specificity for RNA degradation is transmissible between silenced and non-silenced plants, but not between silenced plants. *Plant J.* 21: 1-8.
- Settles, M.W. and T.A. Steeves, 1990. Inflorescence development in *Amelanchier alnifolia*. *Can. K. Bot.* 68: 1680-1688.
- Visser, T., 1964. Juvenile phase and growth of apple and pear seedlings. *Euphytica* 13: 119-129.
- Visser, T., 1965. On the inheritance of the juvenile period in apple. *Euphytica* 14: 125-134.
- Visser, T., 1976. A comparison of apple and pear seedlings with reference to the juvenile period. II. Mode of inheritance. *Euphytica* 25: 339-342.
- Visser, T., J.J. Verhaegh, and D.P. Devries, 1976. A comparison of apple and pear seedlings with reference to the juvenile period. I. Seedling growth and yield. *Euphytica* 25: 343-351.
- Weller, J.L., I.C. Murfet, and J.B. Reid, 1997. Pea mutants with reduced sensitivity to far-red light define an important role for phytochrome A in day-length detection. *Plant Physiol.* 114: 1225-1236.
- Westwood, M.N., 1970. Rootstock-scion relationships in hardiness of deciduous fruit trees. *HortScience* 5(5): 418-421.
- Westwood, M.N., 1993. Temperate-zone pomology: physiology and culture. 3rd ed. Timber Press, Portland, Oregon.
- Wood, B.W. and C.C. Reilly, 2001. Atypical symptoms of cold damage to pecan. *HortScience* 36(2): 298-301.
- Zatylny, A.M., J.T.A. Proctor and J.A. Sullivan, 1996. Assessing cold hardiness of red raspberry genotypes in the laboratory and field. *J. Amer. Soc. Hort. Sci.* 121(3): 495-500.
- Zimmerman, R.H., D.T. Krizek, W.A. Bailey and H.H. Klueter, 1970. Growth of crabapple seedlings in controlled environments: Influence of seedling age and CO₂ content of the atmosphere. *J. Amer. Soc. Hort. Sci.* 95(3): 323-325.
- Zimmerman, R.H., 1971. Flowering in crabapple seedlings: methods of shortening the juvenile phase. *J. Amer. Soc. Hort. Sci.* 96(4): 404-411.
- Zimmerman, R. H., 1972. Juvenility and flowering in woody plants: a review. *HortScience* 7(5): 447-453.
- Zimmerman, R.H., 1977. Relation of pear seedling size to length of the juvenile period. *J. Amer. Soc. Hort. Sci.* 102(4): 443-447.

